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Clonal spread of invasive *Ludwigia hexapetala* and *L. grandiflora* in freshwater wetlands of California

Miki Okada [a,*], Brenda J. Grewell [b], Marie Jasieniuk [a]

[a] Department of Plant Sciences, Mail Stop 4, University of California, Davis, CA 95616, USA
[b] USDA-ARS Exotic and Invasive Weeds Research Unit, Department of Plant Sciences, Mail Stop 4, University of California, Davis, CA 95616, USA

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ABSTRACT

Ludwigia hexapetala and *L. grandiflora* are recent, aggressive invaders of freshwater wetlands in California. To assess the relative role of sexual versus clonal reproduction in invasive spread, we used AFLP markers to genotype 794 ramets of *L. hexapetala* sampled from 27 populations in three watersheds of California, and 150 ramets of *L. grandiflora* from five populations in a fourth watershed. We then used two analytical methods, similarity thresholds and character incompatibilities, to distinguish genotypic variation within genets (clones) from variation between genets. Our analyses revealed extremely limited genotypic and genet variation in invasive *L. hexapetala* and *L. grandiflora* within California. Within *L. hexapetala*, 95% of the ramets analyzed represented a single genet. The genet was the only one detected in 20 populations. The remaining seven populations contained two to nine genets. Within *L. grandiflora*, all ramets were of only one genotype. Thus, invasive spread within and between populations, and across watersheds, appears to be almost exclusively clonal and brought about by the dispersal of vegetative propagules. The extremely low seedling recruitment indicates that management should target vegetative dispersal and growth.

1. Introduction

Aquatic invasive plants in freshwater wetlands spread rapidly within and between populations and can cause substantial losses in native biodiversity and ecosystem function (Cook, 1985; Wade, 1990). The characteristic rapid spatial spread results from the nature of dispersal in aquatic ecosystems. The most potent dispersal agent is the flow of water, including normal water currents and floods (Sculthorpe, 1967). As a result of water dispersal, the spatial distribution of invasive plants often correlates with the hydrological connectivity of suitable habitats within a geographical area (e.g. Shimamura et al., 2007; Thomas et al., 2006; Truscott et al., 2006). Hydrological connectivity also leads to metapopulation structure, which increases the likelihood of population persistence (Levin et al., 2003; Ouborg et al., 1999) and invasion success.

Most angiosperms in aquatic ecosystems have mixed reproductive strategies involving sexual and clonal reproduction with water dispersing both seeds and vegetative propagules, e.g. shoot fragments and bulbils (Cook, 1985, 1987; Grace, 1993). In these aquatic plants, the relative importance of sexual versus asexual reproduction often varies among populations (Barrett et al., 1993; Eckert, 2002; Honnay and Bossuyt, 2005). Variation in reproductive mode has been attributed to constraints on recruitment from seed due to local biotic or abiotic environmental factors, such as intra- and interspecific competition or water depth (e.g. Hangelbroek et al., 2002; Jacquemyn et al., 2006; Kudoh et al., 1999). Genetic factors, including inbreeding depression and polyploidy, also influence the selective advantage of sexual versus clonal reproduction. The extent of clonal versus sexual reproduction within populations in turn affects genet persistence, mating patterns, and genotypic diversity (Barrett et al., 1993; Eriksson, 1993; Grace, 1993; Pan and Price, 2002; Silander, 1985). Assessing the relative importance of sexual versus clonal reproduction within populations, and dispersal of sexual versus clonal propagules among populations, can thus provide insight into the demographic and evolutionary processes underlying successful invasions of aquatic vascular plants. Moreover, information on the spatial pattern of reproduction and dispersal of sexual and clonal propagules can lead to the design of effective strategies for managing aquatic plant invaders (Davies and Sheley, 2007).

Ludwigia hexapetala and *L. grandiflora* are recent, aggressive invaders of freshwater wetlands in California (Cal-IPC, 2006; Wagner et al., 2007; B. Grewell, D. Canington, and J. Futrell, unpublished data). The emergent aquatic perennial plants are found in slow-flowing rivers, at lake and reservoir margins, and in

* Corresponding author. Present address: Western Regional Research Center, U.S. Department of Agriculture-ARS, 800 Buchanan Street, Albany, CA 94710, USA. Tel.: +1 530 752 0508; fax: +1 530 752 4604.
E-mail address: mokada@ucdavis.edu (M. Okada).

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the shallow waters of canals and floodplains. Dense stands have degraded natural communities, reduced water quality and floodwater retention, and prevented effective mosquito control. *L. hexapetala* is currently expanding its range in both northern and southern California whereas, to date, *L. grandiflora* has only been found in the San Diego River and associated wetlands. Both species are capable of asexual (clonal) reproduction by vegetative propagation and sexual reproduction by seed (Hoch, 1993; Ruaux et al., 2009; Wagner et al., 2007). The plants produce creeping submerged stems that root at nodes and produce aerial shoots. Both clonal (vegetative) and sexual propagules are dispersed by water. Floating vegetative mats or shoot fragments readily break off and are carried away by flowing water. Fruits, which contain numerous small and sexually produced seeds, also float in water and are easily dispersed by water currents.

The objective of this study was to assess the relative importance of sexual versus clonal reproduction and propagule dispersal in the invasive spread of *L. hexapetala* and *L. grandiflora* in freshwater wetlands of California. To infer the mode of reproduction underlying population growth within invaded sites, as well as dispersal across the invaded wetland landscape, we quantified multilocus genotypic variation within and between populations of the two species using Amplified Fragment Length Polymorphism (AFLP) markers. We then used two analytical methods, similarity thresholds and character incompatibilities, to distinguish genotypic variation within genets (clones) from variation between genets. Identifying variation among ramets clonally reproduced from a single genet that result from somatic mutations, and distinguishing this genotypic variation from variation among genets, enabled inferences concerning the relative role of sexual versus clonal reproduction and propagules in invasive spread.

2. Materials and methods

2.1. Plant sampling

L. hexapetala was sampled from 27 sites in three California watersheds separated by major geomorphic drainage divides. The watersheds were the: (1) North Coast Watershed (Russian River Basin), (2) Sacramento River Watershed, and (3) San Joaquin River Watershed. Within a watershed, sampling sites consisted of a diversity of wetland types connected by perennial or ephemeral water flow, including rivers, lakes, and floodplains. For comparison, we also sampled two populations of *L. hexapetala* in the Columbia River Watershed of Oregon and Washington.

L. grandiflora was sampled from the one river basin of California where it has been identified, namely, the San Diego River Basin of the South Coast Watershed, which drains into the Pacific Ocean. We sampled *L. grandiflora* at five sites within this watershed. In addition, we sampled *L. peploides* ssp. *peploides* (three populations), *L. peploides* ssp. *montevidensis* (one population), and both subspecies (one population). The two *L. peploides* subspecies were used as taxonomic outgroups for the data analyses because *L. peploides*, *L. hexapetala*, and *L. grandiflora* are all members of *Ludwigia* section *Oligospermum* and the most closely related *Ludwigia* species that occur in California.

At each sampling site, young leaves were collected from 30 randomly chosen shoots greater than 10 m apart from each other, with the exception of sites WSYO and BBBR where 20 and 24 samples, respectively, were collected because *Ludwigia* population sizes were smaller at these locations. Only leaves that developed above water were sampled to avoid DNA contamination from other aquatic organisms. Sampled leaf tissue was placed in plastic zipper bags filled with silica gel (Chase and Hills, 1991) for shipping or transport to the laboratory then frozen and stored in microcentrifuge tubes at −80 °C until DNA extraction.

2.2. DNA extraction and AFLP analysis

Total genomic DNA was extracted from approximately 10 mg of the dried and frozen leaf tissue using a DNeasy Plant Mini Kit (QIAGEN, Valencia, CA). AFLP analyses followed the method of Vos et al. (1995) with minor modifications. Restriction digestion and adapter ligation were performed according to Kim and Rieseberg (1999). Selective pre-amplifications were performed using EcoRI and MseI primers consisting of adapter sequences with a single selective nucleotide (EcoRI + A and MseI + C) in a total volume of 25 μL containing 1 μL of the restriction and ligation reaction as template, 0.2 μM of each primer, 120 μM of each dNTP, 0.5 U Taq polymerase (QIAGEN), and 1× Taq polymerase buffer (QIAGEN). PCR conditions for the pre-amplification reactions were 72 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 2 min, and a final step of 60 °C for 10 min. Selective amplifications were performed using EcoRI and MseI primers with three selective nucleotides in a total volume of 12.5 μL containing 2.5 μL of 1:10 diluted pre-amplification reaction product as template, 0.04 μM of fluorescently labeled EcoRI primer, 0.2 μM of MseI, 0.1 μg bovine serum albumin, 2 mM $MgCl_2$, 0.5 U of Amplitaq Gold Taq polymerase (Applied Biosystems, Foster City, CA), and 1× Taq polymerase buffer (Applied Biosystems). Selective amplifications were performed with an initial denaturation step of 94 °C for 2 min, followed by 13 cycles of 94 °C for 30 s, 65 °C for 30 s with a decrement of 0.7 °C per cycle and 72 °C for 2 min, followed by 24 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 2 min and a final step of 72 °C for 10 min. We used eight primer combinations: EcoRI + ACA/MseI + CGG, EcoRI + ACC/MseI + CCT, EcoRI + AGG/MseI + CAA, EcoRI + AGG/MseI + CGT, EcoRI + AGT/MseI + CAC, EcoRI + ATC/MseI + CAC, EcoRI + ATC/MseI + CCG, and EcoRI + ATG/MseI + CAC) for the selective amplifications. The eight primer pairs were chosen for their polymorphism and ability to produce markers that could be scored unambiguously based on screening 256 EcoRI and MseI primer combinations, each with three selective nucleotides beginning with A and C, respectively. PCR amplified fragments were separated and sized on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and GeneMapper version 3.7 with GeneScan 500 ROX as internal size standard. Fragments of sizes between 50 and 500 bp were scored.

To assess the repeatability of the AFLP results, four separate reactions, including the restriction, ligation, and pre-selective and selective amplification steps were performed using DNA from 26 randomly selected individuals. The reactions, which were repeated four times for each of the 26 individuals, produced identical AFLP profiles. In addition, because most (95.3%) samples of *L. hexapetala* had the same multilocus profile, analyses were repeated for all samples that differed from this predominant multilocus genotype. For the samples that differed in AFLP profile, DNA was re-extracted from the dried and frozen leaf tissue, and the restriction, ligation, and pre-selective and selective amplifications repeated. Similarly, all *L. peploides* samples with markers in low frequency, i.e. with a frequency <0.1 within a population, were rescored following a separate AFLP analysis performed on DNA re-extracted from leaf tissue. Loci with non-repeatable markers were omitted from the data analysis, as were AFLP loci that were monomorphic across all samples in the study. Based on the AFLP profiles, six of the 30 samples assumed to be *L. peploides* ssp. *peploides* in one population were found to be *L. hexapetala*. These samples were excluded from data analyses.

2.3. Data analysis

To estimate genotypic diversity within each population, we calculated the total number of genotypes (G), the effective number of genotypes (G_E), and Simpson's diversity index (G_D) corrected for

sample size using the software GENOTYPE/GENODIVE version 1.1 (Meirmans and Van Tienderen, 2004). G_E is equal to the total number of genotypes (G) when all genotypes have the same frequency; otherwise, G_E is less than G. Simpson's diversity index, G_D, is the probability of sampling two distinct genotypes within a population. G_D varies from zero, when all individuals in a population have the same genotype, to one, when each individual has a distinct genotype.

Genets were identified using similarity thresholds based on the frequency distribution of pairwise marker distances between each ramet sampled within a population (Douhovnikoff and Dodd, 2003) as implemented in GENOTYPE/GENODIVE (Meirmans and Van Tienderen, 2004). Based on this analysis, threshold marker differences of one and two were chosen for *L. hexapetala* and *L. peploides*, respectively, to assign each genotype to a genet. We then estimated genet diversity in terms of the total number of genets (g), the effective number of genets (g_E), and Simpson's diversity index corrected for sample size (g_D).

To detect recombination as evidence of sexual reproduction in clonal organisms that also reproduce sexually, we used the character incompatibility method of Anderson and Kohn (1998) and Mes (1998). Within all pairwise comparisons of individuals for a binary character or marker, no more than three of the four possible marker comparisons (0/0, 1/0, 0/1, and 1/1) should be present within ramets of a single genet, assuming no parallel or reverse mutations. Presence of all four combinations of binary markers signifies character incompatibility and indicates recombination as the source of genotypic variation in the sample. The analysis was performed on each of the populations that had more than four distinct multilocus genotypes. For *L. hexapetala*, we also ran the analysis on all unique genotypes detected. We calculated matrix incompatibility (the total number of pairs of characters that are incompatible in a data set) using the program jactax.exe in the PICA 4.0 software package (Wilkinson, 2001). In addition, we examined whether most or only a few genotypes in the data set caused incompatibilities by successively deleting individuals responsible for the greatest number of incompatibilities following Van Der Hulst et al. (2000). If incompatibilities are caused by only a few individuals, successive deletion results in a sharp decrease of matrix incompatibility. On the other hand, if each individual is a distinct genet, successive deletion results in a gradual decrease of matrix incompatibility until only four multilocus genotypes are left in the analysis. Individuals were successively deleted from the analysis until the matrix incompatibility became zero or only four distinct multilocus genotypes remained in the data set.

To visualize the relationships among genets within and between populations, a UPGMA dendrogram of all distinct genets detected within each population was constructed based on the distance coefficient of Nei and Li (1979) with 1000 bootstrap replications using PAUP* version 4 (Swofford, 2002).

3. Results

The eight selective primer pairs yielded 235 polymorphic loci that could be unambiguously scored for the 1154 ramets analyzed in this study. A total of 64 monomorphic loci were also scored but not used in the data analyses. Of the 235 polymorphic loci, the presence of an allele at 138 loci was species specific. Alleles were shared by at least two species for the other 97 polymorphic loci.

In *L. hexapetala*, 813 (95.2%) of the 854 ramets analyzed shared a single multilocus AFLP genotype (Table 1). This predominant genotype was characterized by the presence of a DNA fragment, and thus a peak in the electropherograms, at 119 loci. The remaining 41 ramets (4.8% of the total) differed from the predominant genotype at four or more of 52 of the 119 loci. In addition, three ramets from populations FRWA1 and LTBC (Table 1) revealed the presence of a peak at a single locus besides the 119 detected in the predominant genotype. In contrast to *L. hexapetala*, all 150 ramets of *L. grandiflora* shared the same multilocus genotype, which was characterized by the presence of a peak (marker) at each of 107 loci.

Within the populations sampled, we detected no genotypic variation in 20 of the 29 populations of *L. hexapetala* (Table 1). In two of the remaining nine populations, FRWA1 and LTBC, the histogram of pairwise distances between samples showed a unimodal distribution (Fig. 1) indicating a mutational origin of the genotypic variation observed. Moreover, the two ramets from population FRWA1 that differed in genotype had the same AFLP mutation, strongly suggesting clonal spread subsequent to mutation. The remaining seven populations with multiple genotypes showed a bimodal distribution of pairwise distances among the plants sampled (Fig. 1), which indicates sexual recombination and thus recruitment from seed. Using the method of Meirmans and Van Tienderen (2004), we detected a total of 15 distinct genets, in addition to the predominant genet, across the seven populations, WSYO, LKWI, UBLS, FRWA4, FRWA5, RRSB, and FRWA6 (Fig. 1). Population UBLS had the largest genet diversity with nine distinct genets (Table 1). Five of the genets were identified multiple times in three to nine plant samples from the population whereas the remaining four genets were observed only once. The genet that was predominant in all other populations was only detected in one sample.

Consistent with the analysis of individual pairwise distances for population UBLS (Fig. 1), 228 matrix incompatibilities were detected among unique genotypes from this population (Fig. 2). In a separate analysis performed on all 18 unique genotypes detected within *L. hexapetala*, the matrix incompatibility count was 679. Matrix incompatibilities decreased gradually upon successive deletion of genotypes causing the greatest number of incompatibilities in the data set in both analyses, indicating that the genotypic variation detected resulted from recombination due to sexual reproduction.

For *L. peploides*, 86 AFLP loci were polymorphic in the 144 ramets sampled from five populations. The number of multilocus AFLP genotypes detected within each population ranged from three in LKHE to 26 in SPLK (Table 1). No identical multilocus genotype was shared between any *L. peploides* populations. A unimodal distribution of pairwise distances among plant samples in populations WLND, LKCL, and LKHE (Fig. 1) indicates that each population consists of a single genet with genotypic variation of mutational origin. In contrast, the frequency distribution of pairwise distances in SNWR appeared narrowly bimodal while population SPLK showed at least two peaks in the frequency distribution of pairwise distances, indicating the presence of genotypes originating from seed recruitment.

There were no character incompatibilities among the seven multilocus genotypes found within WLND, LKCL, and LKHE. Matrix incompatibility counts in SNWR and SPLK were 19 and 378, respectively (Fig. 2), indicating the presence of genotypic variation originating from recombination due to sexual reproduction. The matrix incompatibilities gradually decreased with successive deletion of ramets. No incompatibilities were detected after deletion of four AFLP genotypes in SNWR and deletion of 16 genotypes in SPLK.

In the UPGMA dendrogram (Fig. 3), all distinct genets of the three *Ludwigia* species formed two major clusters, each with 100% bootstrap support. One of the two clusters consisted of genets of *L. peploides* whereas the second, larger cluster included both *L. hexapetala* and *L. grandiflora* genets. Within this large cluster, all distinct genets of *L. hexapetala* and *L. grandiflora* clustered according to their species identity with 100% bootstrap support (Fig. 3). Within the *L. hexapetala* group (Fig. 3), genets did not

Table 1
Genotypic and genet (clonal) diversity in *Ludwigia hexapetala*, *L. grandiflora*, and *L. peploides*, as assessed using AFLP markers and GENOTYPE/GENODIVE software (Meirmans and Van Tienderen, 2004). Wetland habitat type refers to riverine (R), palustrine lakeshore (L), palustrine floodplain (F), or managed seasonal pond (S). N, sample size; H, habitat type; G, number of genotypes; G_E, effective number of genotypes; G_D, genotypic diversity; g, number of genets; g_E, effective number of genets; g_D, genet diversity. Genet diversity measures are indicated in parentheses unless they equal genotypic diversity values.

Taxon[a]/watershed/population	Location	Latitude (°N)	Longitude (°W)	N	H	$G(g)$	$G_E(g_E)$	$G_D(g_D)$
L. hexapetala								
North Coast								
RRCS	Russian River @ Comminsky Creek	38.89	123.06	30	R	1	1.00	0.00
RRAB	Russian River @ Asti	38.77	122.99	30	R	1	1.00	0.00
RRHE	Russian River @ Healdsburg	38.77	122.86	30	R	1	1.00	0.00
LKWI	Lake Wilson	38.52	122.86	30	L	2	1.30	0.24
RRSB	Russian River @ Sunset Beach	38.50	122.93	30	R	2	1.07	0.07
RRWO	Russian River @ Wohler Pool	38.51	122.88	30	R	1	1.00	0.00
LSLR	Laguna de Santa Rosa @ Laguna Ranch	38.45	122.84	30	F	1	1.00	0.00
LTBC	Laguna de Santa Rosa @ Blucher Creek	38.38	122.78	30	R	2(1)	1.07(1.00)	0.07(0.00)
Sacramento River								
UBLS	Upper Butte Basin @ Llano Seco	39.55	121.92	30	S	9	5.11	0.83
UBDC	Upper Butte Basin @ Little Dry Creek	39.36	121.88	30	S	1	1.00	0.00
BBBR	Butte Basin @ Behring Ranch	39.34	121.93	24	S	1	1.00	0.00
CNWR	Colusa National Wildlife Refuge	39.19	122.05	30	S	1	1.00	0.00
CBDY1	Colusa Basin Drain Yolo @ Knights Landing	38.81	121.78	30	R	1	1.00	0.00
CBDY2	Colusa Basin Drain @ Yolo County Line	38.92	121.91	30	R	1	1.00	0.00
WSYO	Willow Slough Canal, Yolo 96	38.58	121.84	20	R	2	1.10	0.10
YBWA	Yolo Bypass Wildlife Area	38.56	121.64	30	S	1	1.00	0.00
WLND	Winchester Lake @ North Delta	38.44	121.57	30	L	1	1.00	0.00
FRWA1	Feather River @ Oroville Wildlife Area	39.45	121.64	30	R	2(1)	1.14(1.00)	0.13(0.00)
FRWA2	Feather River @ Oroville Wildlife Area	39.45	121.60	30	F	1	1.00	0.00
FRWA3	Feather River @ Oroville Wildlife Area	39.45	121.61	30	F	1	1.00	0.00
FRWA4	Feather River @ Oroville Wildlife Area	39.44	121.64	30	F	2	1.07	0.07
FRWA5	Feather River @ Oroville Wildlife Area	39.43	121.63	30	F	2	1.07	0.07
FRWA6	Feather River @ Oroville Wildlife Area	39.42	121.62	30	L	3	1.15	0.13
San Joaquin River								
CRAR	Cosumnes River @ Arnold Road	38.32	121.33	30	F	1	1.00	0.00
CRDR	Cosumnes River @ Dillar Road	38.34	121.33	30	F	1	1.00	0.00
CRRR	Cosumnes River @ Riley Road	38.35	121.30	30	F	1	1.00	0.00
CRTR	Cosumnes River @ Twin Cities Road	38.29	121.38	30	R	1	1.00	0.00
Columbia River								
WASS	Solo Slough @ Longview	46.16	123.02	30	R	1	1.00	0.00
OREU	Golden Gardens Pond @ Eugene	44.09	123.18	30	L	1	1.00	0.00
L. grandiflora								
South Coast								
SDMT	San Diego River @ Mission Trails Park	32.84	117.03	30	R	1	1.00	0.00
SDKP	San Diego River @ Kaiser Pond	32.77	117.14	30	R	1	1.00	0.00
SDFV	San Diego River @ Fashion Valley	32.76	117.17	30	R	1	1.00	0.00
SDMP	San Diego River @ Mitigation Ponds	32.76	117.19	30	R	1	1.00	0.00
SDPY	San Diego River @ Mission Preserve	32.76	117.19	30	R	1	1.00	0.00
L. peploides ssp. *peploides*								
Sacramento River								
WLND	Winchester Lake @ North Sacramento Delta	38.44	121.57	30	L	4(1)	1.30(1.00)	0.24(0.00)
SNWR	Sacramento NWR, Willows	39.43	122.19	30	S	16(3)	6.25(1.15)	0.87(0.13)
North Coast								
LKCL	Lake Cleone, McKerricher State Park	39.49	123.79	30	L	7(1)	2.14(1.00)	0.55(0.00)
SPLK	Spring Lake, Santa Rosa	38.46	122.65	30	L	26(11)	22.50(4.09)	0.99(0.78)
L. peploides ssp. *montevidensis*								
San Francisco Bay								
LKHE	Lake Hennessey, Napa	38.49	122.35	30	L	3(1)	1.15(1.00)	0.13(0.00)
North Coast								
SPLK	Spring Lake, Santa Rosa	38.46	122.65	30	L	26(11)	22.50(4.09)	0.99(0.78)

[a] Taxon identity according to B. Grewell, D. Canington, and J. Futrell (unpublished data).

cluster with high bootstrap support, with the exception of three genets from population UBLS, which clustered with 94% bootstrap support. The genets of *L. grandiflora* sampled from the five sites formed a single highly supported cluster in agreement with their species identity and the lack of genotypic variation detected (Fig. 3). Genets of *L. peploides* formed two well supported clusters, each with 95% bootstrap support, corresponding to subspecies *peploides* in populations WLND, LKCL, and SNWR, and subspecies *montevidensis* in LKHE. Genets from population SPLK were found in both *L. peploides* clusters indicating the presence of both subspecies in the population.

4. Discussion

Our analyses using AFLP markers revealed extremely limited genotypic variation in invasive *L. hexapetala* and *L. grandiflora* in freshwater wetlands of California. Thus, invasive spread of the two species within and between populations, and across watersheds, appears to be almost exclusively clonal and brought about by the dispersal of vegetative propagules. The limited genotypic variation detected was mostly attributable to sexual reproduction rather than somatic mutations within ramets of a genet, in agreement with the recent introduction of *L. hexapetala* and *L.*

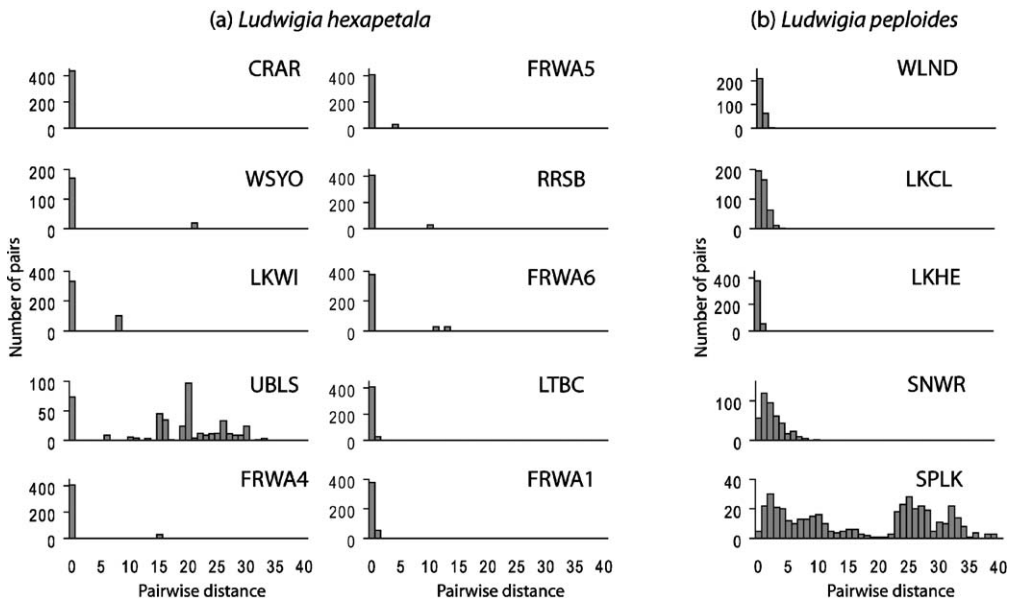

Fig. 1. Frequency distributions of the number of pairs of individuals at each pairwise distance: (a) within the nine populations of *Ludwigia hexapetala* in which AFLP genotypic variation was detected and, for comparison, within a population at the Cosumnes River, CRAR, in which no genotypic variation was detected and (b) within each population of *L. peploides*. The frequency distribution is multimodal if there are ramets belonging to multiple genets within the population. The first peak close to zero represents small within-genet genotypic differences arising from mutations or rare genotyping artifacts whereas the second peak represents between-genet genotypic differences arising from recombination. Pairwise distances were calculated using GENOTYPE/GENODIVE (Meirmans and Van Tienderen, 2004).

grandiflora into the watersheds sampled in California (Cal-IPC, 2006).

Our results are consistent with the major role that vegetative propagules have generally been found to play in the long distance dispersal of aquatic clonal plants in contrast to terrestrial clonal species (Pyšek, 1997). In terrestrial clonal plants, seeds are the primary means of long distance dispersal whereas vegetative propagules mainly contribute to local population growth (Silander, 1985; Stebbins, 1950). In contrast, in aquatic environments, vegetative propagules are commonly dispersed within and between populations by water currents and animals (Boedeltje et al., 2003; Cook, 1987; Figuerola and Green, 2002; Thomas et al., 2005). As vectors of dispersal, water currents, in particular, have the capacity to generate high propagule pressure. In *L. hexapetala* and *L. grandiflora*, such water dispersal of shoot fragments and/or floating vegetative mats, especially from large existing stands, may contribute to both population growth and invasive spread. Furthermore, in *L. hexapetala*, the lack of genotypic variation among watersheds suggests that vegetative propagules have been responsible for overland dispersal of the invasive species between discrete watersheds. Shoot fragments of other aquatic weeds have previously been shown to be transported overland by birds or human transfer of boats among watersheds (Johnson et al., 2001; Johnstone et al., 1985). In Washington state, *L. hexapetala* previously was sold as a water garden plant in nurseries (Hamel and Parsons, 2001). Clonally propagated plants may therefore also have been dispersed via the ornamental trade.

This study shows that recruitment from vegetative propagules predominates in invasive populations of *L. hexapetala* and *L. grandiflora* in California and that seedling recruitment is very rare. Seedling recruitment in clonal plant species has been conceptualized to vary between two extremes related to environmental disturbance and intraspecific competition (Eriksson, 1989, 1993). At one extreme in disturbed areas, it is thought to occur only during initial colonization, followed by vegetative reproduction during population growth. At the other extreme, repeated seedling recruitment is thought to occur during population growth because intraspecific competition does not suppress seedling development. In addition to being influenced by disturbance and competition, seedling recruitment in aquatic plants may also depend on the water regime, which affects the deposition of seeds in the soil as well as their germination (e.g. Nathan and Muller-Landau, 2000; Nicol et al., 2003; Warwick and Brock, 2003). A canopy cover created by other species in the community may also reduce seedling recruitment (Lindig-Cisneros and Zedler, 2002; Maurer and Zedler, 2002). In this study, population UBLS exhibited notably greater genet variation than other populations of *L. hexapetala*, indicating more seedling recruitment in this population. UBLS is a population in a newly restored wetland area with much open space. Environmental disturbance associated with restoration activities may have promoted seedling recruitment at this site by not only suppressing intraspecific competition but also by increasing the probability of deposition of floating fruits with seeds, providing relief from inundation for germinating seeds, and/

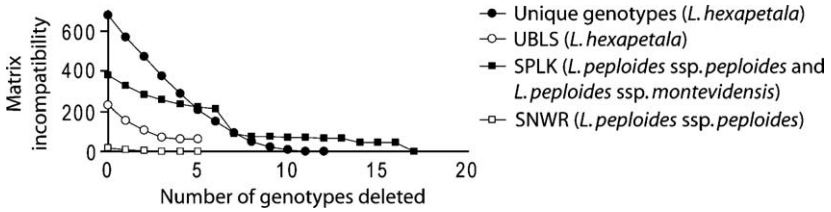

Fig. 2. Reduction of matrix incompatibilities after successive deletion of the unique AFLP genotypes detected in all the ramets of *Ludwigia hexapetala* analyzed, and within three populations (UBLS, SPLK and SNWR) in which greater than four genotypes were detected. Matrix incompatibilities were calculated using PICA version 4.0 (Wilkinson, 2001).

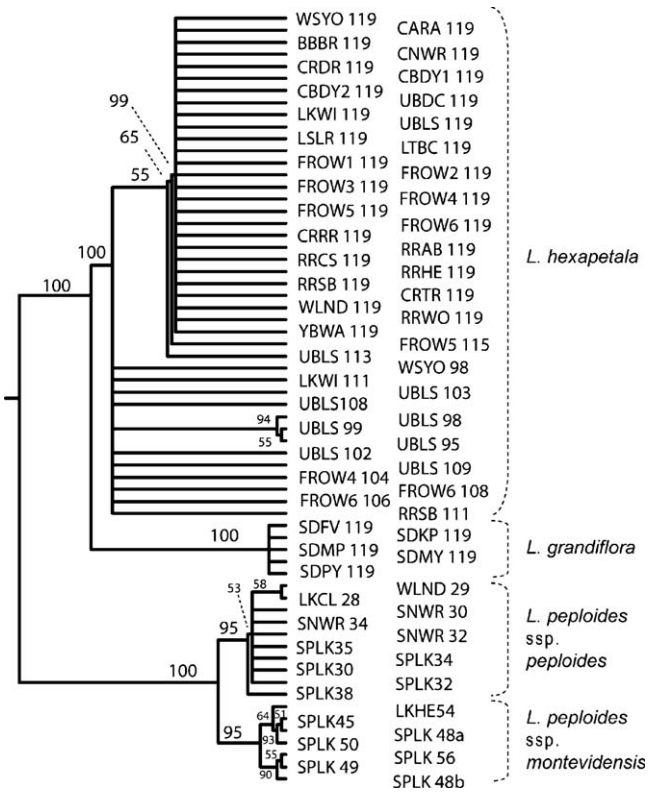

Fig. 3. Dendrogram generated from UPGMA cluster analysis of pairwise distances (Nei and Li, 1979) among distinct AFLP genotypes of *L. hexapetala*, *L. grandiflora*, and *L. peploides* in California, USA. Numbers next to the population IDs indicate the number of DNA fragments (markers) detected for each genotype.

or reducing canopy cover. Spot treatment of newly colonizing patches of *L. hexapetala* with herbicide during the preceding growing season (Steven Cordes, pers. comm.) may also have increased seedling recruitment by reducing intraspecific competition.

In addition, genetic factors could potentially limit seed production and seedling recruitment. Inbreeding depression has been shown to result from pollination within or between flowers of the same plant in clonal outcrossing species (Eckert, 2002; Reusch, 2001; Travis et al., 2004). Although the mating system of *L. hexapetala* or *L. grandiflora* has not been investigated (Raven, 1979), *L. hexapetala* is protandrous and appears to require pollinators to set fruit (Dandelot et al., 2005), which suggests outcrossing. Thus, inbreeding depression could potentially limit seed production and seedling recruitment in California populations of these invasive *Ludwigia* species.

The two invasive populations WASS and OREU of *L. hexapetala* from the Columbia River basin in Washington and Oregon (Table 1) consisted of the same clone as the predominant clone detected in invasive populations of California. Since WASS is known to have resulted from releases of ornamental plants (Sytsma et al., 2004), the predominant clone in California and OREU may share an ornamental origin with the one identified in Washington and not result from an independent introduction from the native range.

Invasive populations of aquatic plant species often consist of a single clone (e.g. Hofstra et al., 2000; Hollingsworth and Bailey, 2000; Wang et al., 2005) or only a few clones (Ren and Zhang, 2007) indicating spread by vegetative propagules. A general lack of seedling recruitment, as observed in this study, has also been reported in many aquatic invasives (Eckert et al., 2003; Larson, 2007; Lui et al., 2005). Low genetic diversity resulting from the dispersal of vegetative propagules and clonal reproduction in introduced ranges is disadvantageous for the long-term evolutionary potential of invasive populations. However, such dispersal and reproduction can be of short-term demographic advantage when a single propagule or a few propagules found a new population following long-distance dispersal (Barrett and Richardson, 1986). In addition, the rate of expansion of a clonal species in an introduced range may be increased by rapid propagation of hybrid or especially invasive genotypes (Moody and Les, 2002; Pyšek et al., 2003) or clonal integration between ramets (e.g. Oborny et al., 2000; Xiao et al., 2007). Phenotypic plasticity, rather than ecotypic variation, may maintain fitness in varying environments across the range of invasion by a clonal invasive plant (Baker, 1965; Geng et al., 2007; Richards et al., 2006).

Our results indicate that the management, including biological control, of invasive *L. hexapetala* and *L. grandiflora* populations should focus on controlling vegetative growth and the dispersal of shoot fragments under the current hydrological regime. However, populations may be driven toward sexual reproduction rather than clonal reproduction under other managed hydrological schemes, such as drawdown. Under such management that promotes seedling recruitment, biological control agents that attack the sexual reproductive process may also be needed to slow invasive spread.

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